## Escherichia coli Field Contamination of Pecan Nuts

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More pecan samples collected from grazed orchards were contaminated with *Escherichia coli* than were samples from nongrazed orchards. No differences in frequency of contamination between mechanically and manually harvested nuts occurred. Nutmeats from whole uncracked pecans that were soaked for 24 h in a lactose broth solution containing *E. coli* did not become contaminated. Twenty-four percent of the whole pecans soaked in water for 48 h to simulate standing in a rain puddle developed openings along shell suture lines which did not completely close when the nuts were redried.

Nutmeats as a potential source of microbial contamination of foodstuffs was not reported until 1927 when Weinzirl (12) found that candies containing nuts had more bacterial contamination than candies without nuts. In a later study (13) he indicated that the nutmeats were the source of the contamination, but attributed this primarily to unsanitary practices by the vendors handling the nuts. Further investigation revealed that, insofar as pecans were concerned, there was no one area in pecan processing that could be specifically cited as a contamination trouble spot, but that the entire process. involving tempering, cracking, shelling, storage, and handling, contributed to contamination (3, 9, 10, 11). Standard methods for decontamination of nuts at shelling plants were proposed (11).

It has been shown that some Escherichia coli on walnut hulls remain through the tempering process and subsequently contaminate the nutmeats (8). The walnut industry is becoming aware of the potential hazard caused by maintaining livestock in orchards (1). Chipley and Heaton (4) obtained shelled pecan samples from commerical operations and found that 20% of them tested positive for E. coli. This is of direct concern both to the producer, as evidenced by Food and Drug Administration seizures of such contaminated pecans (2), and to the consumer, as pecans are often eaten raw or entered raw into already cooked foods such as candy. Kokal and Thorpe (7) followed the E. coli contamination levels of almonds throughout the growing season and found that the level began to rise during the mechanical harvesting operation and peaked during the precleaning process. These processes are similar to those employed in pecan harvesting, which began to change to mechanical harvesting in the 1960s. This, coupled with the widespread practice of grazing cattle in pecan orchards, added a new dimension to problems of nut contamination by introduction of fecal material. We observed clumps of dried manure in some bins of mechanically harvested nuts from grazed orchards. In general, cattle are removed from pecan orchards several weeks prior to harvest. Sometimes the land is further prepared for mechanical harvest by disking the vegetation and manure under and smoothing the land. However, more often the grass cover is maintained as a permanent pasture and is therefore only mowed prior to harvest. Currently there is no testing or control of the amount or type of microbial contamination present on in-shell pecans before they enter the shelling plants.

The objectives of this study were to compare the degree of *E. coli* contamination on nuts harvested from grazed and nongrazed orchards and to investigate some related problems in the orchard at harvest time. Samples were collected over a 2-year period. The 1970 harvest season was considered wet, and the 1971 season was considered dry.

## MATERIALS AND METHODS

E. coli on pecans from grazed versus nongrazed orchards. Pecans were aseptically collected in sterile Whirl-pak bags (7.5 by 3 inches; 19.05 by 7.62 cm) during the harvest season from grazed and nongrazed pecan orchards in Georgia and Alabama. One sample

per orchard in 1970 and five random samples from each orchard in 1971 were obtained. Each sample consisted of 100 to 150 g of in-shell pecans, out of which 50 g were rinsed in sterile buffer by the method of Hyndman (6). Serial dilutions ranging from 1 to 10<sup>-5</sup> ml were made of the washings, inoculated into tubes of lauryl sulfate tryptose broth (LST), and incubated at 35 C for 48 h. After 24 and 48 h of incubation. all samples showing gas production were inoculated into tubes of EC medium and incubated at 45.5 C (5). Gas-positive samples after 48 h of incubation were inoculated into eosin methylene blue agar plates and incubated at 35 C. Different morphological type colonies on the eosin methylene blue plates were tested for indole-methyl red-Voges Proskauer-citrate (IMVIC) reactions.

Penetration of unbroken shells by E. coli. Five replications of five nuts each of the pecan cultivar Cape Fear, having no visible cracks, were placed in lactose broth inoculated with E. coli. After 24 h at room temperature, the nuts were removed, surface sterilized in a 0.1% mercuric chloride solution, rinsed three times with distilled water, and aseptically cracked. Pieces of nutmeat and middle septum were removed and placed directly into tubes of LST broth. A sterile cotton swab was moistened in sterile buffer, wiped across the inside surface of the shell, and then placed in LST broth. These tubes were incubated at 35 C and observed for gas formation after 24 and 48 h.

Formation of cracks and induced suture opening. Five replications of five pecans each were placed in 500-ml Erlenmeyer flasks containing 200 ml of water. The flasks were capped to prevent evaporation, left at room temperature, and observed daily for the development of cracks or openings along the shell sutures.

## RESULTS AND DISCUSSION

E. coli on pecans from grazed versus non-grazed orchards. There were about six times as many E. coli-contaminated samples collected from grazed orchards than from ungrazed ones (Table 1). This proportion of contaminated samples was obtained during both the wet (1970) and the dry (1971) years, but the levels of contamination were greater during the wet year, with over one-third of the nuts being contaminated.

A comparison of harvesting methods in these orchards revealed no differences in frequency of contamination between mechanically and manually harvested nuts (Table 2).

Penetration of unbroken shells by E. coli. After 48 h of incubation at 35 C, none of the LST broth tubes containing nutmeat, middle septum, or cotton swabs developed gas, indicating that the bacteria did not move through the intact shells to the pecan kernels.

Formation of cracks and induced suture openings. Of the 25 nuts soaked in water for 1 week, 6 opened along the shell suture lines.

TABLE 1. Comparison of E. coli contamination on in-shell pecans from grazed and nongrazed orchards

Orchards	1970		1971		Ag %
	No. of samples	% with E. coli	No. of samples	% with E. coli	with E. coli
Grazed Nongrazed	11 15	36 6	45 30	20 3	23 4

TABLE 2. Influence of harvest method on E. coli contamination of pecan nuts

Type of harvest	No. of s	amples	% contaminated		
	1970	1971	1970	1971	
Machine Manual	5 21	15 <b>6</b> 0	20 \ 19	6.6 6.6	

None cracked anywhere else on the shell. These openings developed within the first 48 h of soaking, and none subsequently closed completely when the nuts were dried. Nuts sometimes stand in water for several days in rainy periods during the harvest season. In a grazed orchard, cracks induced by water absorption or mechanical harvesting could provide an easy entryway of  $E.\ coli$  penetration to pecan kernels. Cleaning processes employed by growers and shellers do not include removal of cracked but otherwise intact nuts. Such nuts also may not be decontaminated by currently used procedures prior to shelling and subsequently could contaminate shelling equipment.

This study suggests that, even though *E. coli* would not move through unbroken shells of pecan nuts, it is possible for these shells to open along suture lines or be cracked in the field and thereby present an entrance for contamination into the nutmeats. This, in combination with the fact that there is a sixfold increase in contamination on nuts from grazed orchards over those from nongrazed orchards, would indicate that greater care will have to be exercised in pecan orchards than is currently being done to avoid *E. coli* contamination. The eventual permanent removal of cattle from pecan orchards may become a necessity.

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